Supplemental Document

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Super-Resolution-Chip: an *in-vitro* platform that enables super-resolution microscopy of co-cultures and 3D systems: supplement

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Super-Resolution-Chip: An *In-Vitro* Platform that enables Super-Resolution Microscopy of co-cultures and 3D systems

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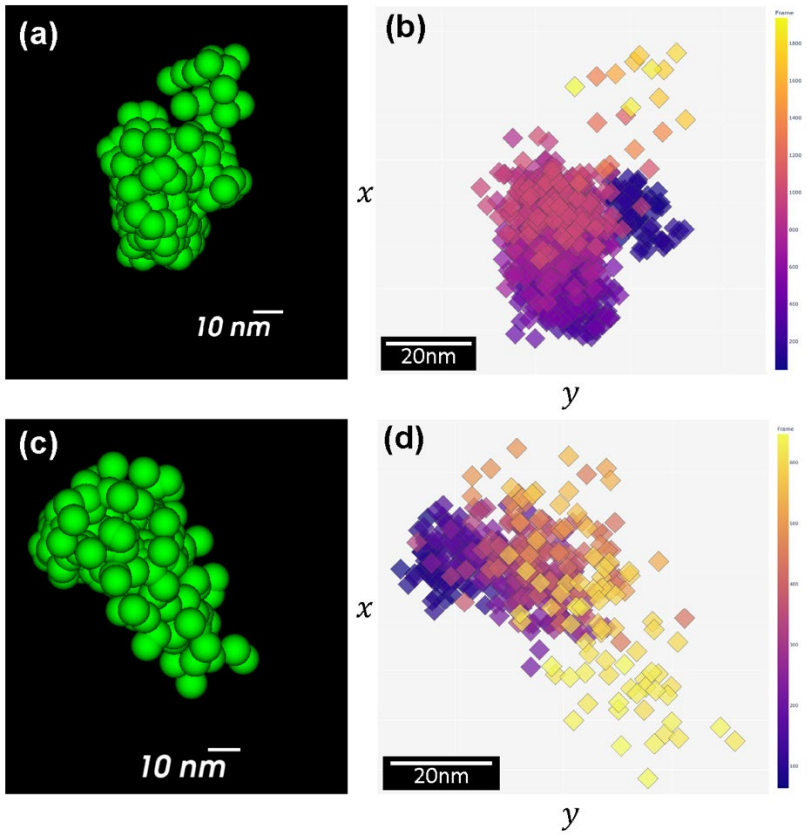
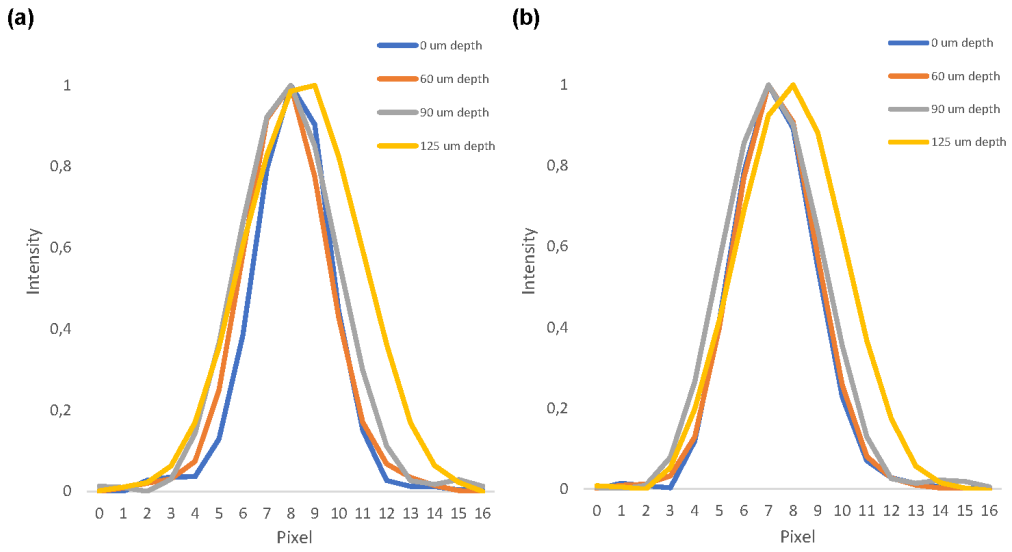
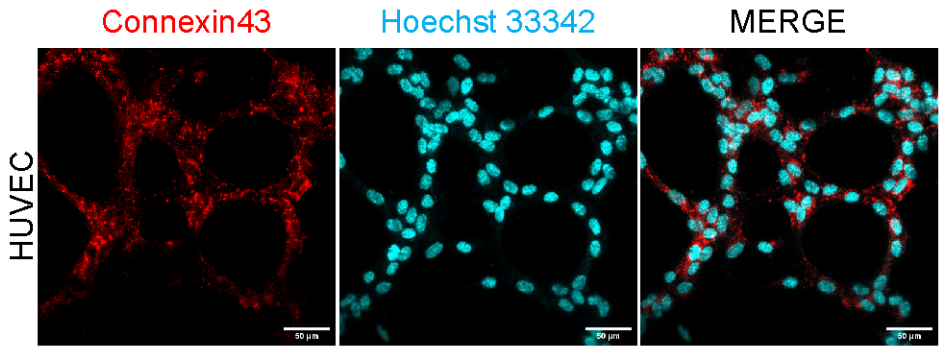
Supplemental Figures:



Supplemental Fig. 1. Drift measurement of 100 nm beads using dSTORM. Tetraspeck™ beads were mounted on glass (1.5H), imaged with a 561 laser at 30% for 2000 frames. (a, c) Two examples of beads imaged for 2000 frame. Each localization is shown as green dot. (b, d) To visualize drift, each localization from the images (a, c) were coloured according to the frame in which it was identified demonstrating a small drift in the XY axis from the beginning of the movie (dark blue color) to the end of the movie (yellowish color).



Supplement Fig. 2. Biplane normalized PSF at various depths of field. Tetraspeck™ beads of 100 nm were imaged at different depth to examine possible distortion of the PSF. As can be seen from the normalized PSFs, none of the PSFs were distorted while the PSF imaged at 125um depth was wider, suggesting slightly lower precesion. Pixel size = 96 nm. (a) Plane 1 (b) Plane 2. The distance between the two planes is 100 nm.



Supplemental Fig. 3. Confocal images of cells cultured in a layer of matrigel. Confocal reconstruction of HUVEC monoculture stained for Connexin43 (red) and nuclei (cyan). Endothelial cells show the formation of tubular structures.

Supplemental Movies:

Visualization 1. Representative dSTORM blinking video. A typical dSTORM raw scan 2000 frames. Each "cutout" seen in the video is later fitted with a point spread function (PSF) to recreate localization point cloud with high precision. The movie shows 6.99 frames per second.

Visualization 2. Confocal reconstruction of cultures grown on both size of the membrane. Z-stack reconstructions of HUVEC and U87 cells grown on both sides of the membrane.